6-PHOSPHOGLUCONATE DEHYDROGENASE FROM SHEEP LIVER: INHIBITION OF THE CATALYTIC ACTIVITY BY FRUCTOSE-1,6-DIPHOSPHATE*

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SUMMARY

The catalytic activity of 6-phosphogluconate dehydrogenase, isolated from sheep liver, has been found to be markedly inhibited by fructose-1,6-diphosphate (F-1,6-PP). Activity measurements carried out in the presence and absence of F-1,6-PP indicate that the inhibition is competitive with respect to 6-phosphogluconate (6-PG), and non-competitive with respect to TPN. A K_i value of 7.08 ± 3.10 x 10^{-5} M for F-1,6-PP may be calculated from the kinetic measurements. As the K_m for 6-PG (1.5 x 10^{-5} M), the K_i for F-1,6-PP, and the concentrations of 6-PG and F-1,6-PP in liver, are all comparable, it appears that the inhibition of 6-phosphogluconate dehydrogenase by F-1,6-PP may be of significance in the regulation of carbohydrate metabolism in the liver.

It has been shown recently by Brown and Wittenberger (1) that F-1,6-PP inhibits the catalytic activity of 6-phosphogluconate dehydrogenase from Streptococcus faecalis. McLean and Gumaa (2) also have data indicating a similar inhibition in the case of the enzyme from rat liver. As we have recently established a method for isolating pure 6-phosphogluconate dehydrogenase from sheep liver (Dyson, Hanson, D'Orazio and Hatlelid, manuscript in preparation) it seemed of interest to test whether the enzyme from this source was also subject to inhibition by F-1,6-PP. Preliminary experiments (Fig. 1A) showed that F-1,6-PP was indeed a potent inhibitor of sheep liver 6-phosphogluconate dehydrogenase, so a detailed investigation of the kinetics of the inhibition has been carried out, the results of which are reported here.

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MATERIALS AND METHODS

TPN, sodium salt, 6-PG, trisodium salt, and F-1,6-PP, trisodium salt, were purchased from Sigma Chemical Company. Solutions of substrates and inhibitors, which were prepared in deionized, glass-distilled water, were standardized by enzymatic assay assuming a molecular absorptivity of 6.22×10^3 (3). Reaction velocities were determined by following the rate of reduction of TPN at 340 nm with a Beckman DB-G recording spectrophotometer. 6-Phosphogluconate dehydrogenase[4] was isolated from sheep liver (Dyson, Hanson, D'Orazio and Hatlelid, manuscript in preparation) obtained from local slaughter houses. The preparations used had specific activities of 15 to 17 units per mg protein at 30° and pH 7.7, and were homogeneous according to the criteria of the analytical ultracentrifuge and isoelectric focusing. One unit of enzyme is defined as that amount of enzyme which will convert 1 μmole of substrate to product in 1 minute at 30° and pH 7.7. All rate measurements were carried out in 0.05 M tris/acetate buffer adjusted to 0.10 ionic strength with KCl, and at pH 7.7 unless otherwise indicated, and at a temperature of 30°.

RESULTS

Determination of the pH value at which optimum inhibition occurred showed it to be essentially identical with the pH value for optimum enzyme activity (Fig. 1B), kinetic studies were therefore carried out at pH 7.7. Below pH 6.0, and above pH 8.8, little inhibition could be detected (Fig. 1B).

Data from enzyme activity measurements, carried out as a function of 6-PG concentration, in the presence and absence of F-1,6-PP, is presented in Fig. 2B, plotted in a double reciprocal manner. The common intercept on the 1/v axis for experiments with and without inhibitor indicates that the inhibition is competitive with respect to 6-PG. From the values from K_m and K_{app} obtained from the intercepts on the $1/[6\text{-PG}]$ axis of Fig. 2B, together with the concentrations of inhibitor used, the K_i for F-1,6-PP may be calculated to be 9.2×10^{-5} M.

[4] 6-Phospho-D-gluconate: NADP oxidoreductase (decarboxylating) EC 1.1.1.44

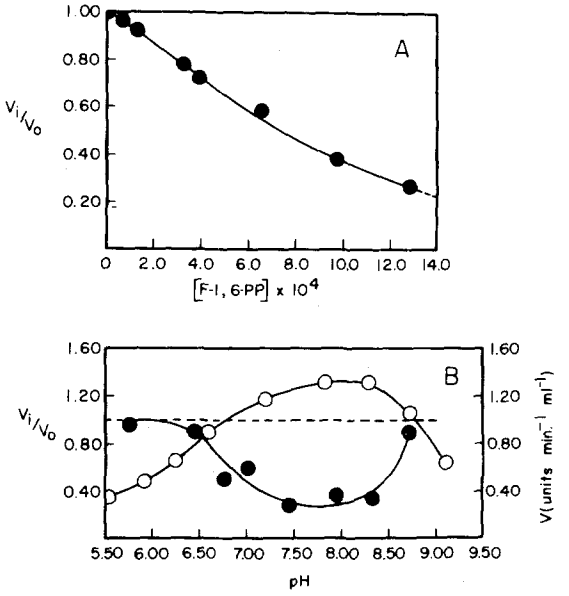
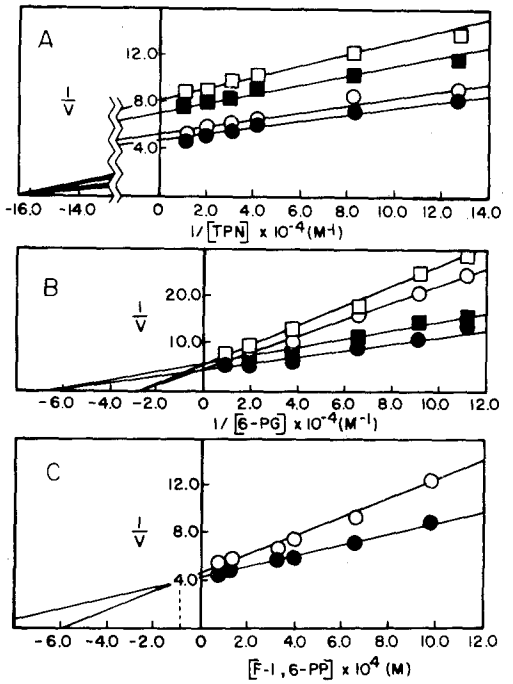


Fig. 1A. Degree of inhibition as a function of F-1,6-PP concentration at constant substrate levels. 6PG concentration 5.28×10^{-5} M; TPN concentration 1.54×10^{-4} M. Fig. 1B. O—O Effect of pH on the activity of 6-phosphogluconate dehydrogenase (right hand ordinate). 6PG concentration 4.28×10^{-4} M; TPN concentration 3.70×10^{-4} M. ●—● Effect of pH on the degree of inhibition by F-1,6-PP (left hand ordinate). 6PG concentration 5.36×10^{-5} M; TPN concentration 1.54×10^{-4} M; F-1,6-PP concentration 1.29×10^{-4} M. Other conditions of Figs. 1A and 1B as indicated under Materials and Methods.

In Fig. 2A data from enzyme activity measurements, carried out as a function of TPN concentration, in the presence and absence of F-1,6-PP, is presented as a double reciprocal plot. In contrast to the measurements carried out as a function of 6-PG concentration the common intercept in this case occurs on the $1/[\overline{TPN}]$ axis, thus the effect of the inhibitor is on the velocity of the reaction, not on the affinity of the enzyme for TPN, indicating that the inhibition is non-competitive with respect to TPN. After correction for lack of saturation of the enzyme by 6-PG, values for V_{max} and V_{app} may be obtained from Fig. 2A. From these values, together with the concentration of inhibitor used, the K_i for F-1,6-PP may be calculated to be 8.4×10^{-5} M.

The average value for the K_i for F-1,6-PP, calculated from Figs. 2A and 2B, and from a number of other, similar, plots, is $7.08 \pm 3.10 \times 10^{-5}$ M.

As a further check on the nature of the inhibition caused by F-1,6-PP,



Figs. 2A and 2B. Effect of F-1,6-PP on the activity of 6-phosphogluconate dehydrogenase as a function of substrate concentration. Activity measurements were carried out in the presence of: closed symbols, no F-1,6-PP; open symbols, 1.23×10^{-4} M F-1,6-PP. Substrate concentrations. Fig. 2A. ○ and ● 9.05×10^{-5} M 6PG. □ and ■ 1.81×10^{-5} M 6PG. Fig. 2B ○ and ● 1.54×10^{-5} M TPN. □ and ■ 7.70×10^{-5} M TPN. Fig. 2C. Effect of F-1,6-PP on the activity of 6-phosphogluconate dehydrogenase at constant substrate levels (method of Dixon (4)). Substrate concentrations. 1.54×10^{-4} M TPN. ○—○ 1.06×10^{-4} M 6PG. ●—● 5.28×10^{-5} M 6PG. Figs. 2A, 2B and 2C. Other conditions as described under Materials and Methods.

activity measurements were carried out as a function of F-1,6-PP concentration, at two different concentrations of 6-PG. The data from these measurements is shown in Fig. 2C, plotted according to the method of Dixon (4). Lines drawn through the points obtained from the measurements carried out at the two different 6-PG concentrations intersect at a point well above the /F-1,6-PP/ axis indicating, in support of the data of Fig. 2B, that the inhibition is competitive with respect to binding of 6-PG. A K_i of 8.0×10^{-5} M may also be obtained directly from this point of intersection, in good agreement with the value obtained from the double reciprocal plots.

DISCUSSION

A comparison of the structure of 6-PG and F-1,6-PP shows a certain similarity insofar as both molecules have a negative charge at each end of the molecule The negative charge at the C1 end of the molecules appears to be a necessary factor for binding to the enzyme, since glucose-6-phosphate and fructose-6-phosphate have been tested for inhibition and found to have no measurable effect on the rate of catalysis by 6-phosphogluconate dehydrogenase. It will be noted from Fig. 1B that the efficiency of F-1,6-PP as an inhibitor of 6-phosphogluconate dehydrogenase activity follows quite closely the pH-activity profile of the enzyme. The measurements presented in Figs. 2B and 2C also show that F-1,6-PP is competitive with respect to 6-PG binding to the enzyme. This data, together with the similarity in structure of the two molecules, supports the possibility that F-1,6-PP is binding at the active site of the enzyme. In contrast to the results of Brown and Wittenberger (1) our results do not show an effect of F-1,6-PP on the K_m for TPN.

Although data on the levels of 6-PG and F-1,6-PP in sheep liver is not available, measurements of the levels of these metabolites in rat liver have been made. Arese (5) indicates that the concentration of 6-PG in rat liver is of the order of 0.027 μmole/g. wet weight liver (approximately 3×10^{-5} M). Hohorst et al (6) present data showing that the concentration of F-1,6-PP in rat liver is 0.017 to 0.03 μmole/g. wet weight liver (approximately 2 to 3 $\times 10^{-5}$ M). Moreover, the K_m for 6-PG (1.5×10^{-5} M) and the K_i for F-1,6-PP (7.08×10^{-5} M) are not dissimilar. If similar concentrations of 6-PG and F-1,6-PP occur in other species, then inhibition of 6-phosphogluconate dehydrogenase by F-1,6-PP may well be of significance from the regulatory viewpoint in controlling the entrance of glucose-6-phosphate into the pentose phosphate pathway. It is, perhaps, of significance that both phosphofructokinase (7) and pyruvate kinase (8) are activated by F-1,6-PP, thus increased levels of F-1,6-PP would increase the activity of these two enzymes, while limiting the levels of glucose-6-phosphate entering the pentose phosphate pathway.

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